EFFECT OF LIGHT ON SOLANINE SYNTHESIS IN THE POTATO TUBER

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(WITH SIX FIGURES)

In 1820 DESFOSSES (**15**) discovered solanine, a basic substance in juice expressed from the berries of *Solanum nigrum*. He found (**14**) it almost insoluble in all solvents tried, except alcohol and acids. Six years later BAUP reported (**5**) it also to be present in the potato (*Solanum tuberosum*), but he found much more in the sprouts than in the tubers.

The chemical nature of the substance is even yet only partially known. ZWENGER and KIND (**51**) found that it is stable when boiled with potassium hydroxide, and that it cannot reduce Fehling's solution, but (**50**) that it can be hydrolized by strong acids, giving rise to salts of an alkaloid (solanidine) and a solution which is able to reduce Fehling's solution. Identification of the hydrolytic products has been a gradual achievement of several investigators. It appears (**24**) that a molecule of solanine is composed of one molecule each of solanidine, glucose, galactose, and rhamnose; and that three molecules of water are split out in the formation of the glucoside whose constituent groups are linked in the order in which they have been mentioned (**35, 49**). The empirical formula of solanidine is still a matter of disagreement (**6, 35, 42, 46, 49**), but it is known to be a tertiary base with a secondary alcohol group (**42, 46**).

The glucoside is interesting for several reasons:

1. It is the only basic-reacting glucoside known (**31**).[1]
2. It has the foam-producing and haemolytic properties of saponins, yet contains nitrogen.
3. It frequently occurs in poisonous concentrations in market potatoes.
4. It is claimed that lesser concentrations increase intestinal absorption (**28**) by precipitating sterols (?).
5. It is uniquely toxic to conidiospores of *Cladosporium fulvum* which have been raised on glucose agar (**1, 41**) although spores taken directly from infected tomato leaves tolerate it better.
6. It contains two sugar groups that have not been found free in the plant. If the availability of rhamnose or galactose, or both, limits the elaboration of solanine then a study of the factors which increase the amount of glucoside formed may indirectly contribute to an understanding of how monosaccharides, other than glucose, arise.

[1] Since neither solaneine, nor any glucoside of solanthrene has yet been isolated in crystalline form, the hypothetical solanine group of glucosides is here referred to as "solanine."

To determine in what tissue the solanine is formed and stored, a number in the parenchyma and around the fibro-vascular bundles. By allowing the the most commonly employed procedures. Using such a method, ALBO reports (**3**: p. 199) that solanine is found relatively in abundance in active meristems of all parts of the plant, and that it disappears from inactive tissues. In the tuber he claims that it is

"particularly localized in the periderm (phellogen), although it is also found in the parenchyma and around the fibro-vascular bundles. By allowing the tubers to sprout, it is found very diffusive in the sprouts, and it is also observed in the pith and in the subepidermal cells of the roots."

All such color tests involve the use of concentrated sulphuric acid (**2**), usually warmed. Under such conditions solanine is partially hydrolized to solanidine. Since solanidine also gives these color reactions, HANSEN concluded that such tests cannot be reliable. Color changes of anthocyanins when acid is added offer further complications.

To minimize these difficulties BREHMER (**9**) tried preliminary washing of cut sections with water, ether, and alcohol. Such a method should reduce the troubles from undesired colors, but it also allows leaching out of solanine. HANSEN tried to measure solanine by its haemolytic activity, but was not able to get quantitative results. FISCHER (**18**) showed that the rate of haemolysis varies greatly with the change in the acidity of the medium. Using buffered blood-gelatin strips a quasi-quantitative micromethod was developed by FISCHER and THIELE (**19**) which indicates solanine, as distinct from solanidine. They report no solanine in the periderm of the tuber, most of it being in the ten outer layers of cells of the storage tissue, but at any depth they find more near a sprout than farther away. In the sprouts they find both solanidine and solanine in the region outside the cambium, but only about $\frac{1}{8}$ as much solanine and no solanidine in the central portion. They conclude that solanidine is first elaborated in an actively growing tissue and is later condensed with sugars to form solanine.

MORGENSTERN made a notable advance in gravimetric methods (**34**). His procedure involves extraction of a sample of ground tissue with water and warming the extract with acetic acid. After the filtrate has been evaporated to a syrup, hot alcohol is added. The filtrate is then evaporated to dryness, and is leached with hot acetic acid water. The filtered solution is heated with an excess of ammonium hydroxide. The precipitate is leached with hot alcohol, and the filtrate evaporated to dryness. The residue is dissolved in hot acetic acid water, and the solanine in the filtrate is precipitated with ammonium hydroxide and collected on weighed filter paper.

BÖMER and MATTIS have modified the procedure of MORGENSTERN somewhat. They recommend (**8**):

1. Extracting the ground sample four times with acetic acid water and combining the extracts.

2. Heating the extract with an excess of ammonium hydroxide and evaporating to dryness with diatomaceous earth.

3. Extracting the residue with three or four aliquots of boiling alcohol, alternated with repulverizing in a mortar.

4. Filtering the combined extract and evaporating to dryness.

5. Dissolving the residue in dilute acid and boiling the solution with an excess of ammonium hydroxide.

6. Filtering off and dissolving in hot alcohol.

7. Evaporating the filtrate to dryness and dissolving in dilute acid.

8. Reprecipitating with hot ammonium hydroxide and collecting on a weighed filter paper.

Analytical considerations

Gravimetric methods are tedious and involve so many manipulations that loss of solanine is almost inevitable, in preparing a white precipitate. In a tissue bearing both glucoside and free alkaloid, each will be extracted and the weight of the final precipitate will represent the sum of solanine plus solanidine.[2] Since considerable amounts of the free alkaloid may occur in potato tissue (JORRISSEN and GROSJEAN report (**26**) up to 0.15 per cent. in fresh sprouts), it would seem that a method of analysis would be desirable which would distinguish between the two substances and which would yield more accurate results than the blood, gelatin method of FISCHER and THIELE.

In solanine analysis, either hot ethyl alcohol or, more commonly, a dilute acid is used for extraction. Precipitation is accomplished by evaporation, or by addition of alkali. In these manipulations solanidine and certain magnesium and calcium impurities behave similarly to solanine, making purification possible only at the expense of loss of the product desired for weighing. Weighing introduces further difficulties, for solanine dries to constant weight very slowly. (A 5.3544-gm. sample of Merck solanine spread in a weighing bottle less than one centimeter deep dried to constant weight in an 80° C. oven at one-half atmospheric pressure only after twelve weeks. Moisture content 10.0 per cent.) A large number of filtrations is objectionable for aqueous solutions which have been made alkaline, since excess of alkali, and other factors not completely known, tend to produce a colloidal form of solanine which passes through the filter paper, or at best to produce a gel which clogs the paper, making each process tedious, and often requiring several papers for one filtration.

[2] To verify this, solanidine was prepared from Merck solanine by hydrolysis with 2 per cent. hydrochloric acid and decomposition of the crystalline hydrochloride with alcoholic potassium hydroxide. The base, recrystallized from ether, was found to be well soluble in alcohol and in 0.2 per cent. acetic acid although slowly so at room temperatures.

Extractions can be much simplified by allowing acid rather thoroughly to leach the ground tissue and then pressing quite dry in a hydraulic press. For this purpose a cylinder from an automobile engine, containing two piston heads (the upper one bearing a connecting rod and the other cut off at the second ring and perforated with several holes on the top) makes a satisfactory press cylinder, the pressed material being held in a linen bag between the pistons. A portion of the cylinder head provides support for the lower piston and drainage for the juice through a sparkplug hole into a beaker beneath. By this means it is possible to get out in one leaching practically all of the solanine extractible by the four separate processes of the BÖMER and MATTIS method.

To simplify the procedure and to avoid estimating solanidine as solanine, an attempt was made to estimate the extracted solanine volumetrically, obviating the necessity for weighing, and, more important, the necessity for eliminating the contaminating compounds whose solubilities are so like that of solanine. This is possible where solanine is estimated from the amount of sugar which is split off by acid hydrolysis, for none of these contaminants affect alkaline cupric ion. Because of the small amounts of sugar available in any one analysis, it was found expedient to use a modification of the PAVY reagent (**36**) with methylene blue as an internal indicator (**30**) to sharpen the end point. The alkalinity was increased in order to maintain at least one-half normal sodium hydroxide after addition of all the sugar solution, as seems advisable from the work of QUISUMBING and THOMAS (**38**), to keep a high ratio of cuprous oxide to sugar. The new procedure is simple and expeditious, save for the first filtration, which may be somewhat slow.

In the separation of tuberin from solanine, it is necessary that no solanine should be hydrolized by heating in dilute acetic acid. In a test of this, samples of one-half gram were boiled for one-half hour in 25 cc. of 0.2 per cent. acetic acid. No appreciable reducing ability developed, a confirmation of the work of ZWENGER and KIND (**51**), and of BÖMER and MATTIS (**8**). Indeed, solanine is unique in the conditions requisite for significant hydrolysis. COLOMBANO (**12**) claimed no appreciable hydrolysis at room temperatures for this concentration of solanine in 2 per cent. hydrochloric acid, even after a month's standing. The writer finds that after standing five months only 23 mg. of "glucose" appears under these conditions from a 500-mg. sample. In contrast, heating with 2 per cent. hydrochloric acid gives a fairly complete and rapid hydrolysis. HEIDUSCHKA and SIEGER (**24**) find over three-fourths of the solanine hydrolized in one-half hour at the boiling point.

Analytical procedure

A sample of not much more than one kilogram of fresh tissue is ground in a Russwin mill, and the juice and solid material just covered by 0.2 per

cent. acetic acid. After standing for about six hours, the liquid is strained through a linen bag, the residue being pressed out in a Carver hydraulic press, using a pressure of more than 35 kilograms per square centimeter. This expressed fluid is heated on a water bath to precipitate the tuberin, filtered through paper pulp, the pulp washed out with a few more cubic centimeters of acid, and the filtrate made alkaline by a few drops of ammonium hydroxide. This on further warming precipitates the solanine. It should be left in the evaporating dish overnight to allow large flocs to form. These are collected on a filter paper, in a Büchner funnel using suction, and washed with a few cubic centimeters of water containing a few drops of ammonium hydroxide. The volume of the filtrate is determined and the weight of solanine which it contains is computed, using the value 25 milligrams per liter (**29**).

For estimation, the precipitate is leached through the paper into a 50-ml. volumetric flask, by 25 ml. of 2 per cent. hydrochloric acid, and kept at a gentle boil on a hot plate for one hour. While hot, 10 ml. of 5 per cent. ammonia water is added, and after cooling, the whole is made up to 50 ml. with water. The reducing power of the filtered solution is determined by copper reduction.

The copper reagent consists of three grams of copper sulphate pentahydrate dissolved in 600 ml. of concentrated ammonium hydroxide, added to 1,000 ml. of Fehling's B, and the whole made up to 2,000 ml. with water. Such a solution shows no auto-reduction on standing. A 10-ml. aliquot is raised to boiling in a 300-ml. Erlenmeyer flask, with a drop of 1 per cent. aqueous solution of methylene blue. The flask is fitted with a two-holed rubber stopper, one hole for steam exit, and the other for the offset tip of a burette which is used to run in the sugar solution (the hydrolysate). Titration is complete on loss of the red color.[3] These titration values were standardized, using oven-dried samples of Merck solanine. Using the proportions indicated, at least 15 mg. of solanine should be present in the precipitate. Otherwise, the sugar solution formed is such a weak reducing agent that a large amount of solution is necessary to complete the titration. As it runs into the flask a significant amount of oxygen may also enter. Preferably a precipitate should contain less than one-half gram of solanine because of the danger that more concentrated hydrolysates may bump during the boiling. As shown by figure 1, intermediate values (20–100 milligrams) can be determined with greater accuracy.

Total solanine is obtained by adding the amount precipitated to the amount left in the alkaline filtrate, unprecipitated.

[3] The presence of ammonium ion in the sugar solution, added *during* the boiling, avoids one of the difficulties in the PAVY method, namely, precipitation of cuprous oxide if boiling is prolonged enough to drive off too much ammonia before the titration is complete.

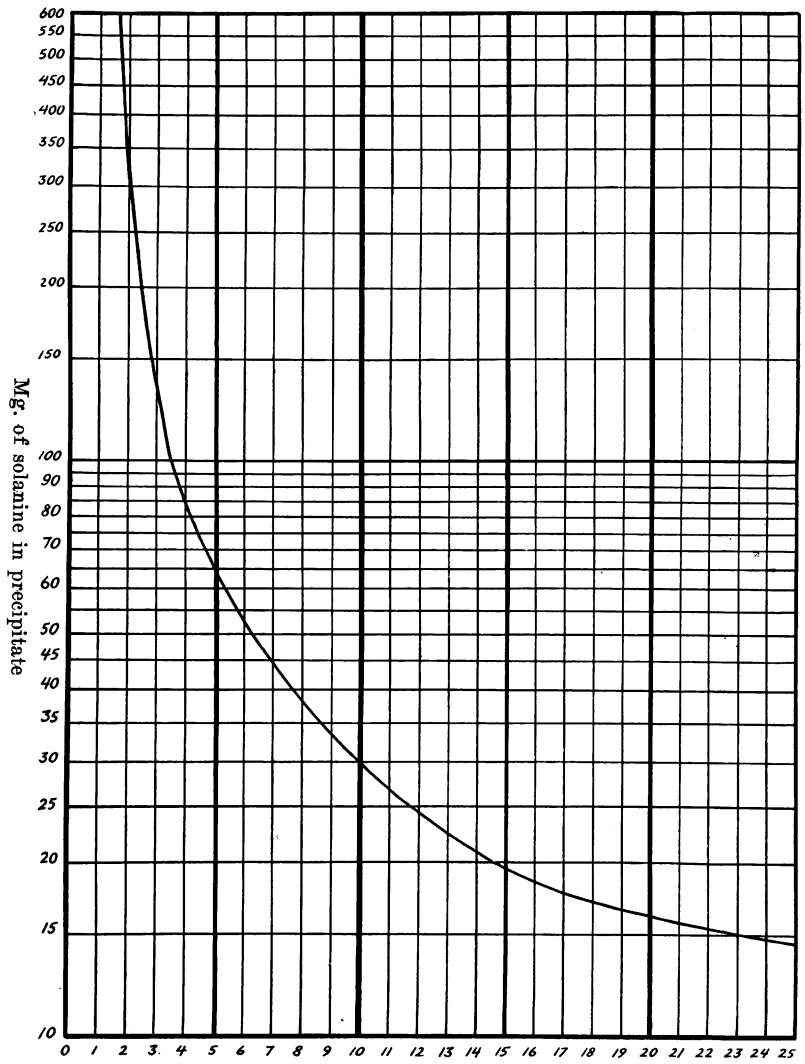

FIG. 1. Copper reduction values of solanine hydrolysates.

Effect of light on tubers

Since solanine-rich tubers are frequently green, and since "sunburned" tubers are uniformly bitter and have an increased solanine content, the matter of tuber irradiation has received the attention of several investigators (**7, 19, 21, 22, 34, 43**). MATTIS found (**7**) that the metabolic activity was quite significant. Old tubers in storage showed very little response to

irradiation, while fresh tubers, when plowed out and exposed to direct sunlight, increased up to more than ten times their previous "solanin" content.

No investigation has been reported which has considered the relative significance of the various wave length bands of sunlight for solanine formation. Since the initial rise in solanine content commonly precedes visible chlorophyll synthesis (**21, 22**) it might be inferred that the former is not a consequence of the latter, but any convincing demonstration must involve the isolation of a selective radiation as a result of which no greening accompanies solanine elaboration. This paper describes an attempt:

(1) to increase the solanine content of tubers without chlorophyll elaboration; and

(2) to determine whether under equivalent intensities the wave lengths which chlorophyll ordinarily utilizes in glucose synthesis are the ones which are most effective in solanine synthesis.

EQUIPMENT

Seven bottomless wooden boxes were constructed, 33×33 cm., 18 cm. high; the side walls were covered with white lead enamel, a good reflector for wave lengths from 0.3 μ to well beyond the visible (**32**: p. 88). The top of each box consisted of a set of four identical Corning glass filters, 16.2×16.2 cm., whose margins were sealed with adhesive tape. An area of approximately 850 sq. cm. remained exposed to the radiation of the tungsten lamp overhead. These seven filters showed transmission peaks in various parts of the spectrum. The transmissions of these glasses were determined over the entire wave length range up to 4 μ, using a quartz spectrograph with sector attachment for the ultra-violet, a spectrophotometer for the visible, and a spectrometer with rock salt prism and thermopile for the infra-red.

Three additional boxes were prepared of sheet iron, 38×48 cm., 30 cm. high, with tops which were tight-fitting removable trays 8 cm. deep. The bottom of each tray had a rectangular cutout 32×42 cm. A sheet of ultra-violet transmitting glass was fastened into each tray bottom with pitch. A cooling coil of copper tubing, carrying a stream of tap water, was fitted just inside the tray wall. The coil and the metal portion of the tray were given two dippings in melted paraffin. Care was taken not to let paraffin overlap more than about 3 mm. onto the clear portion of the glass. Curved false bottoms were placed in each of the three boxes and two of the boxes were placed on platforms of unequal height, so that while the three box bottoms were horizontal, they were at sufficiently different heights to keep the three false bottoms as portions of one large cylindrical surface of 30-cm. radius, whose axis was the tube of an overhead Cooper-Hewitt mercury arc in Uviol glass, which had to be kept on a downslope for proper operation.

TABLE I

CHARACTERISTICS OF THE FILTERS EMPLOYED WITH MAZDA LAMPS

FILTER			LAMP		RADIATION	
DESIGNATION	MEAN THICKNESS	APPROXIMATE TRANSMISSION LIMITS	DISTANCE	WATTAGE	CHARACTER	DESIGNATION
	mm.	μ	*cm.*			
			53.6	100	Visible approximates artificial lighting but weak in yellow band which distinguishes IV from V; infra-red intense;	I
91B	7.62	0.365–4.2				
			57.8	1000	ultra-violet slight	VIII
			24.5	100	Visible like sunlight;	II
G90A	5.23	0.344–4.0			infra-red considerable;	
			51.8	1000	ultra-violet slight	IX
			29.6	100	Visible largely green;	III
G124JA	4.44	0.356–4.2			infra-red slight; ultra-	
			41.2	1000	violet slight	X
G24	4.27	0.600–4.1	33.4	100	Visible only red and orange; (should be efficient for glucose synthesis); infra-red considerable; ultra-violet absent	IV
G34	3.96	0.520–3.6	44.1	100	Visible red, orange, and yellow (should be efficient for glucose synthesis); infra-red considerable; ultra-violet absent	V
		0.460–0.620	34.0	1000	Visible green; infra-red	VI
G401CZ	4.85	and			intense; ultra-violet	
		1.1–3.6	23.8	1000	absent	XI
		0.350–0.480			Visible blue; infra-red	
G53C	4.75	and	27.0	1000	slight; ultra-violet	VII
		1.5–3.6			relatively intense	

Equal portions of the lamp were partitioned off for each filter by sheets of tin plate, as shown in figure 2.

Into one of these trays was placed a 5-cm. layer of an aqueous solution containing 290 gm. of $NiSO_4 \cdot 6\text{–}7H_2O$, and 83 gm. $CoSO_4 \cdot 7H_2O$ per liter. The second tray contained a 5-cm. layer of an aqueous solution containing

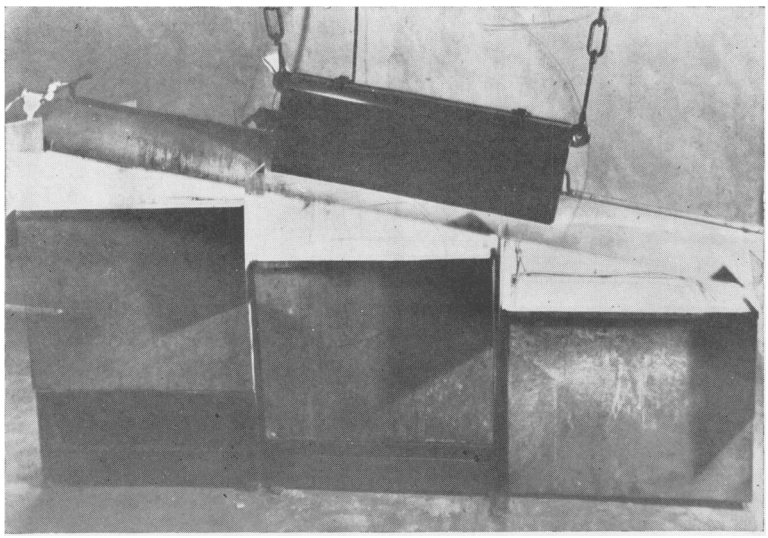

FIG. 2. Mercury arc lamp and metal boxes showing the three tray box tops with cooling coils and the four sheets of tinplate partitioning the lamp.

8.8 gm. $CuSO_4 \cdot 5H_2O$ and 300 cc. of concentrated NH_4OH per liter. The third tray held a 5-cm. layer of an aqueous solution containing 200 gm. of $NaNO_2$ per liter. Radiation entering these three boxes had to pass through the 5-cm. solution layer, the sheet of ultra-violet transmitting glass in the bottom of the tray, and in the case of the Cu filter an additional thickness of ultra-violet transmitting glass which was used as a cover. The transmissions of these filters was computed from measurements made on the solutions and on a sample of the glass, using a quartz spectroscope and a photo-electric cell. Figure 3 indicates the wide dissimilarity in opacity of these three filters. Although available emission data on the 450-watt Cooper-Hewitt arc[4] was limited to that furnished by BUTTOLPH (10) for a new lamp, it may be concluded that tubers under the $-NO_2$ filter received almost no ultra-violet (radiation XIV), tubers under the Cu filter received long wave length ultra-violet and short wave length visible light (radiation XIII), and that the tubers under the Ni-Co filter received no visible radiation but did receive a much more intense exposure to wave lengths of about 0.3 μ than in the other two cases.

The tungsten lamps employed with the Corning glass filters were standard, internally frosted, 115-volt Mazda type C lamps operated at a potential of 113 volts (mean of a 100 hr. graphic record). When new, the energy distributions of their emissions were approximately as shown in

[4] Previously operated for about 1000 hours.

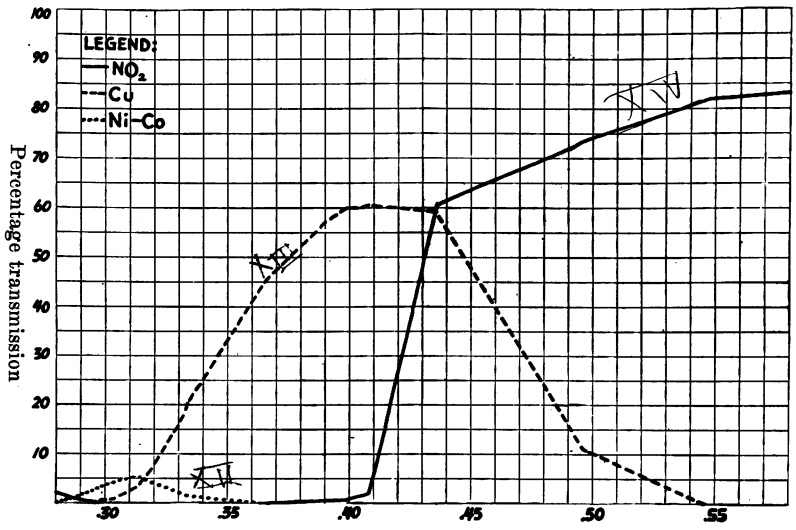

FIG. 3. Transmissions of the filters employed with the mercury arc.

figure 4. With use the emission decreases, but much more rapidly at the short wave length end of the spectrum. To minimize this error in the experiments reported, lamps were used for only 100 hours before replacing with new ones over filters which transmitted below 0.45 μ.

For each wave length the intensity of the lamp source was multiplied by the percentage transmission of the filter in question, and graphed, using a linear scale. Total energy of wave lengths shorter than 0.69 μ was read from this graph, using a planimeter. Knowing that the total radiation of a new 1000-watt lamp was 798 watts, and that of a 100-watt lamp 70.7 watts, and knowing that for the lamps used the intensity of the radiation was about

TABLE II

CHARACTERISTICS OF THE FILTERS EMPLOYED WITH MERCURY ARC LAMP

FILTER		RADIATION	
DESIGNATION	APPROXIMATE TRANSMISSION LIMITS	INTENSITY MAXIMUM	DESIGNATION
	μ	μ	
Ni-Co	0.28 – 0.37	0.30 and 0.31	XII
Cu	0.30 – 0.54	0.43	XIII
$-NO_2$	0.40 – 1.0	0.55	XIV

21 per cent. greater than average, for the cone of light within 48° of the lamp axis (**44**: p. 22), it was possible to compute at what distance a lamp should be placed in order to have any desired intensity on the far side of an interposed glass filter. For computation, the distance from the filament to the center of one of the four glass plates was considered to be the average distance.

For radiations I, II, III, IV, V, VI, and VII these distances were made such that the energy of wave lengths less than 0.69 μ getting through a glass filter would be 100 microwatts per sq. cm. for normal incidence. This

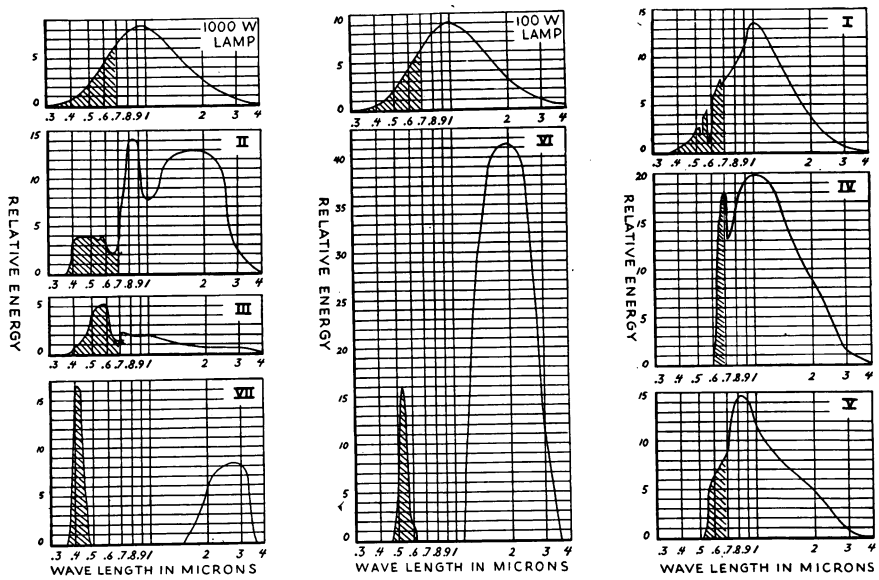

FIG. 4. Spectral energy distributions of radiations I, II, III, IV, V, VI, VII, and of the tungsten lamps employed. The equal energy areas are shaded. Wave lengths are shown on a logarithmic scale in order to make minor differences more apparent in the range which is significant for photosynthesis.

intensity value was chosen quite arbitrarily. The 0.69-μ limit was selected as approximately the upper limit of glucose and chlorophyll synthesis (**13, 37, 39, 40**). Tubers were placed in a single layer on the floor, covered with a filter box, and irradiated from an overhead lamp. Where 1000-watt lamps were employed, the reradiation from the glasses made a 21° ± 2° temperature control at the tuber level possible only after installing compressed air lines to ventilate the boxes.

For radiations VII, VIII, IX, X, and XI, the filter boxes were clustered on edge about a single 1000-watt lamp. In each box the tubers were held in a single layer between two pieces of ½-in. wire mesh, and the box back

was covered with a light-tight pan. Two holes were cut through the bottom side and one in the top for the insertion of elbow pipe fittings so that ventilation could be accomplished through a suction manifold to a vacuum cleaner without entry of extraneous light.

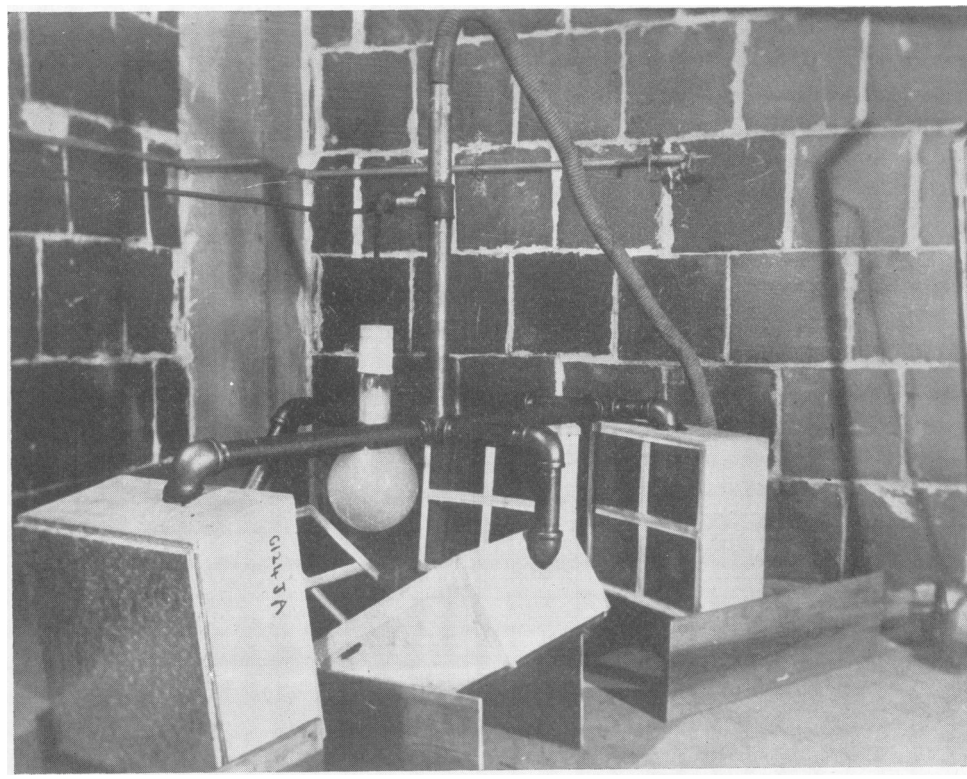

FIG. 5. Arrangement of Corning filters for radiations VII, VIII, IX, X, and XI.

From the results obtained with the previous irradiations, it seemed desirable to compare radiations which were of equal energy in the short wave transmitting band of filter G53C. At the 100-microwatt per sq. cm. intensity this was impractical for filter G401CZ since its transmission in this band (0.35–0.48 μ) was so low that it would have to be placed so close to the lamp that it would subtend too large an angle at the filament (with resultant gross uneven intensities getting through differing portions of the filters). Instead, the G401CZ filter was placed at a distance such that the lower half of its visible transmission band (below 0.53 μ) let through the arbitrary intensity of 100 microwatts per sq. cm.

The angle of incidence of the light rays to the glasses varied from 0° to a maximum of about 46° for the closest placed filter. Within this range the reflection from glass surfaces is uniform (**25**: p. 1572) but the absorption

which occurs in the glass increases with the increasing angle of incidence. Since the transmission measurements were made only for normal incidence, the peaks in figures 4 and 6 should be somewhat more intense than shown.

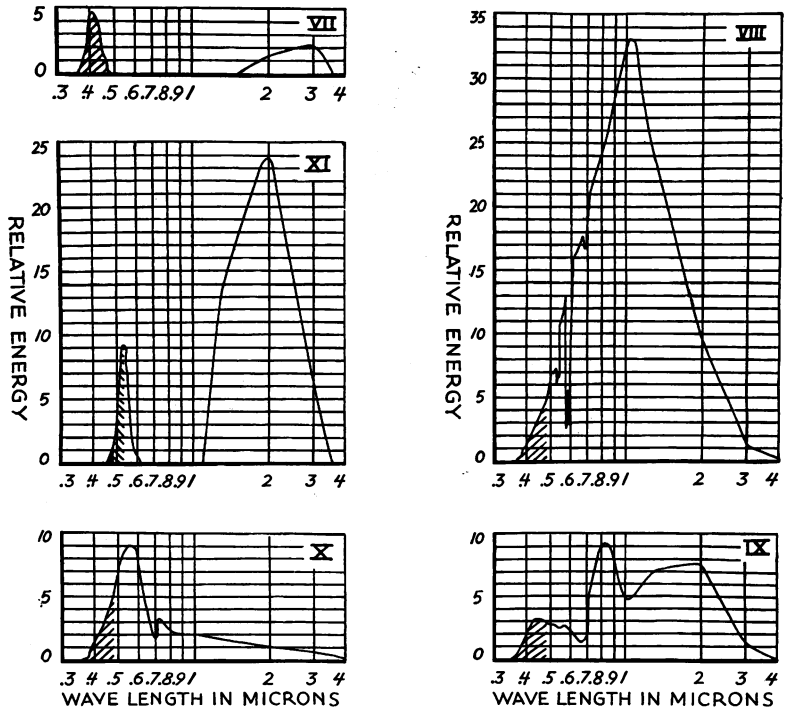

FIG. 6. Spectral energy distributions of radiations VII, VIII, IX, X, XI. The equal energy areas are shaded. Energy units are the equivalent of four of the energy units of figure 4.

Bliss Triumph potatoes were used. The "dormant" tubers were furnished by the State Agricultural Experiment Station at Spooner, Wisconsin. They were dug after sundown, in September, and kept continuously in darkness at room temperature for the two weeks prior to irradiation. This gave some opportunity for storage metabolism to be approximated. The "active" tubers, obtained in May from a dealer at New York Mills, Minnesota, were free from dormacy, having been dug by daylight the previous September and placed in a cool dark storage bin the same day. They were kept in the dark at room temperature for one month, the sprouts off, and the tubers irradiated.

Experimentation

SUNBURNED

"Sunburned" tubers (in which the washing away of soil has exposed them on one side to sunlight for an indefinite number of days) were har-

vested at maturity of the crop and immediately placed in a dark container. After four months' storage in the dark, the short (1–5-cm.) sprouts were removed and the tubers separated into two easily distinguishable portions, green and red, in every case cutting toward the center of the tuber from the boundary of the green zone. Both samples were intensely bitter.

Using the method of analysis described in this paper the solanine contents were found to be as shown in table III.

TABLE III

SOLANINE CONTENT OF SUNBURNED TUBERS AFTER 4 MONTHS OF STORAGE

SAMPLE		SOLANINE			
DESIGNATION	WEIGHT	AMMONIA WATER SOLUBILITY	PRECIPITATE	TOTAL	PERCENTAGE CONCENTRATION
	gm.	*mg.*	*mg.*	*mg.*	%
Red tuber	1001	11	20	31	0.0031
Green tuber	1015	9	24	33	0.0032
Sprouts	995	4	500	504	0.0507

DORMANT

Normal tubers were placed 30 cm. beneath the bare mercury arc lamp for a 20-day continuous exposure. Other tubers were exposed to radiations I, II, III, IV, V, VI, and VII, in each of which the total energy of wave lengths shorter than 0.69 μ was 100 microwatts per sq. cm.

A titration end point was not obtained for the hydrolisates from the precipitates of samples I, II, III, IV, V, VI, and check (indicating less than 10 mg. of solanine in any precipitate).[5] While the exact concentration in these samples remains uncertain, it is certain that the tubers receiving radiation VII contained at least 50 per cent. more solanine than the tubers under any of the other Corning filters. The analytical results are given in table IV.

ACTIVE

Normal tubers were continuously exposed for twenty days to radiations VII, VIII, IX, X, and XI, in each of which the total energy of wave lengths shorter than 0.48 μ was 100 microwatts per sq. cm. Other tubers were given twenty days of exposure to radiations XII, XIII, and XIV. Table V indicates the results obtained.

[5] However, there is no assurance that any precipitate contains solanine unless the titration is complete; the precipitate might be solanidine with calcium and magnesium phosphates only.

TABLE IV

SOLANINE CONTENT OF DORMANT TUBERS AFTER 20 DAYS OF CONTINUOUS RAYING

SAMPLE		SOLANINE			
DESIGNATION	WEIGHT	AMMONIA WATER SOLUBILITY	PRECIPITATE	TOTAL	PERCENTAGE CONCENTRATION
	gm.	*mg.*	*mg.*	*mg.*	%
Bare arc	437	25	22	47	0.0108
Dark (check)	276	5	<10	<15	<0.0026
I	601	7	<10	<17	<0.0028
II	543	4	<10	<14	<0.0026
III	625	3	<10	<13	<0.0021
IV	626	4	<10	<14	<0.0022
V	626	6	<10	<16	<0.0026
VI	799	3	<10	<13	<0.0016
VII	666	5	26	31	0.0047

For the tubers under the last three radiations, color distinctions were marked. Chlorophyll was absent in XII and XIII, and abundant in XIV. Anthocyanin was notable in XII, hardly noticeable in XIII, and not visible in XIV.

TABLE V

SOLANINE CONTENT OF ACTIVE TUBERS AFTER 20 DAYS OF CONTINUOUS RAYING

SAMPLE		SOLANINE				
DESIGNATION	WEIGHT	AMMONIA WATER SOLUBILITY	PRECIPITATE	TOTAL	PERCENTAGE CONCENTRATION	INCREASE OVER CONTROL
	gm.	*mg.*	*mg.*	*mg.*	%	%
Beginning	1725	14	30	44	0.0026	− 7
Dark	1197	6	28	34	0.0028	0
VII	1132	11	48	59	0.0052	+ 85
VIII	1096	10	93	103	0.0094	+236
IX	1128	7	96	103	0.0091	+225
X	1061	6	16	22	0.0021	− 25
XI	1158	5	25	30	0.0026	− 7
XII	1148	10	76	86	0.0076	+171
XIII	1135	13	34	47	0.0041	+ 46
XIV	1197	13	33	46	0.0038	+ 36

Discussion

The concentrations reported here (by the volumetric method) are in general much lower than those obtained by gravimetric estimation of

"Solanin" (including solanidine) (**7, 33, 34**). BÖHME has reported that the normal content of potatoes (not sunburned) may be as much as 0.015 per cent., five times the amount found by the writer in very bitter, sunburned tubers, but BÖHME has reported other normal tubers with concentrations as low as 0.0020 per cent. GORTNER (**20**: p. 466) gives an average value for normal tubers of 0.0024 per cent., which agrees well with the $<$ 0.0026 per cent., 0.0026 per cent., and 0.0028 per cent. values obtained by the volumetric method.

Perhaps more work has been done on anthocyanin formation than on any other type of glucoside synthesis which is induced by radiation. In a recent summary of the field ARTHUR (**4**: p. 1116) concludes:

> "There is a general agreement among various investigators that the blue-violet and often the ultra-violet regions of sunlight are especially effective in anthocyanin production."

Since radiation XII was so much more effective than more intense radiations XIII and XIV, it may be concluded that the ultra-violet band is of more consequence than the blue-violet in the production of potato anthocyanin.

A comparison of qualitatively different light sources by equating total energies gives quite a misleading result where the significant radiations are only a small portion of the total energy, *e.g.*, the visible radiation of a Mazda lamp. This has been a serious error in the studies of KNIEP and MINDER (**48**) and of WURMSER (**13**). By equalizing energies below the 0.69 μ limit, a better comparison was possible as to photosynthetic efficiencies. Greening, and glucose synthesis are most efficient in red-orange light (**13, 47, 48**). Since the most effective increase in solanine occurred upon irradiation by source VII it seems that a glucose synthesis cannot be a necessary intermediate. VII has no red, orange, or short infra-red, but neither has VI. VII lacks green, but so also does IV. The unique formation of relatively large amounts of solanine under radiation VII would not appear to be connected with the omission of any repressant rays, but rather with the presence of rays which induce the synthesis. Of the two possible bands, blue-violet—ultra-violet, and longer infra-red, the latter is ruled out since VII is intermediate in intensity between VI and III as regards these wave lengths.

To test whether the short wave band was responsible, the series of radiations with active tubers was tried. If short rays are effective, then increasing the intensity of I, II, and III to the same level as in this band (VIII, IX, and X) should also show increases in solanine content in tubers that are metabolically active. Whereas radiations I and II did not produce any notable effect on the solanine content of dormant tubers, the same sources were much more effective than VII on active tubers when the inten-

sity was sufficiently greater to give comparable amounts of the short end of the spectrum (VIII, IX). X did not show any increase. Whether green rays have a specific effect in the repression of solanine formation, as induced by short rays, has not been investigated, but the significance of short rays has been confirmed by the results obtained with the mercury arc. XIV contained almost no rays shorter than blue; it induced the least glucoside synthesis. XIII consisted of green, blue, and long ultra-violet; it gave intermediate effect. XII consisted of ultra-violet only, and was much stronger than XIII or XIV below 0.32 μ; it produced the greatest solanine increase. Incidentally it induced no chlorophyll formation.

This study has not been extended to the shorter ultra-violet or to the long infra-red beyond the emission limits of the Mazda lamp since such radiation probably cannot penetrate (**11, 27, 45**) to the tissues in which solanine is formed (**19**). It lumps as "solanine" all substances which precipitate out with the true solanine and which have—or which on acid hydrolysis split off—copper reducing groups. Solaneine (**17**) and the glucoside of solanthrene (**16**) are included, if they occur, but so far as now known they are closely related to solanine in the metabolism of the tuber and they are present only in minor amounts.

Summary

1. A new analytical method has been developed for the quantitative estimation of solanine in the presence of solanidine, based upon the amounts of sugars set free on acid hydrolysis.

2. Upon irradiation by a mercury arc in Uviol or by Mazda lamps, potato tubers increased in solanine. This was accompanied by the appearance of anthocyanin in the sprouts.

3. Wave lengths which are efficient for glucose synthesis did not induce a significant increase in solanine, but did result in an increase of chlorophyll.

4. Ultra-violet rays of about 0.3 μ are effective for solanine formation but not for chlorophyll elaboration.

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